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Changes in Haematological parameters of African Catfish (*Clarias gariepinus*) exposed to sponge plant (*Luffa cylindrica*) leaf extract

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ABSTRACT

The study examined the changes observed in the haematological parameters of African catfish (*Clarias gariepinus*) juveniles exposed to sub-lethal concentrations (883mg/L, 1766mg/L, 3532mg/L, 7063 mg/L and 0.00mg/L as control) of sponge plant (*Luffa cylindrica*) leaf extract using static renewable bioassay for 8 weeks. Two fish from each treatment were sacrificed biweekly and blood samples collected for haematological analysis. During this period, some ecological parameters (pH, Dissolved Oxygen, Temperature, Electric conductivity and Total Dissolved Solids) were monitored. The ecological parameters were relatively stable and were within acceptable limits except for pH which was lower than the recommended range while TDS and conductivity increased with increase in concentration and exposure time. The red blood cell counts, heamoglobin, mean corpuscular volume, platelets and packed cell volumes levels of *C. gariepinus* exposed to sub-lethal concentration of the fruit extract decreased with increase in concentration and exposure time. These decreases were significantly different from that of the control except for mean corpuscular volume ($4.65 \pm 0.21 \times 10^{12}$/L to $2.93 \pm 0.81 \times 10^{12}$/L; 8.53 ± 0.31g/dL to $6.85 \pm 0.1.05$g/dL; $6.04 \pm 0.69 \times 10^{-3}$ to $7.69 \pm 2.19 \times 10^{-3}$; $87.75 \pm 6.34 \times 10^{9}$/L to $58.75 \pm 6.29 \times 10^{9}$/L, 28.00 ± 2.45% to 21.00 ± 3.46% and $3.60 \pm 0.69 \times 10^{9}$/L to 4. $87 \pm 0.18 \times 10^{9}$/L respectively) while the white blood cell counts increased significantly across the different concentrations. This study concludes that, the fruit extract of *L. cylindrica* caused negative changes in the haematological parameters of *C. garienpinus* which can give insight knowledge on the reason for the continuous depletion in our natural fish resources, especially in places where this obnoxious fishing practice is being carried out.

Keywords: Ecological parameters, Haematological parameters, *Luffa cylindrica*, sub-lethal.

INTRODUCTION

Pollution has been defined as any addition or introduction of matter as energy or any substance to the environment in such a quantity that degrades the environment and makes it unfit for humans and other organisms to use or live in. Aquatic pollution is the addition of foreign matter to the aquatic environment to a degree that cannot be supported by the natural system [1]. Pollutants can be grouped into organic (biodegradable) and inorganic (non-biodegradable) pollutants [2]. The contamination of inland water bodies with a wide range of pollutants has become a matter of concern over the last few decades [3].

Botanicals are natural biocides [4] and their contamination of natural waters has become inevitable in Nigeria because of their recent wide use [5]. Piscicidal plants like *Blighia sapida, Tetrapleura tetraptera, Tephrosia vogelii Raphia vinifera, Parkia biglobosa* and *Kigelia africana* are frequently in use by the fisherfolks because they are highly effective [6, 7]. Ekanem *et al*. [8] reported that *Asystasia vogeliana* is highly toxic to the embryos of zebra fish, D*anio rerio*, while Agbon, *et al*. [9] and Omoniyi, *et al*. [10] reported the toxicity of *Nicotiana obaccum* to *Oreochromis niloticus* and *Clarias gariepinus* fingerlings. Water extracts of the floral and barks of *Blighia sapida* and *Kigelia africana* have been reported to be toxic to *Clarias gariepinus* causing increased opercular and tail beats and mortality [11, 12]. Generally, the stressful behaviours of exposed fishes tend to show feelings of respiratory impairment due to direct damage to the gill epithelia, besides accumulation of mucus which may further aggravate the problems [13]. However, the presence of these botanicals in high concentrations may have adverse effects on aquatic organisms.

According to Ekpendu *et al*. [14] *L. cylindrica* ranked among the most commonly used piscicides in many parts of Nigeria.
Luffa cylindrica and *Luffa aegyptiaca* Mill., (2n = 26) commonly called sponge gourd, vegetable sponge, bath sponge or dish cloth gourd, is a member of Cucurbitaceae family [15]. *L. cylindrica* is a wild monoecious wild climbing plant which get to maturity within a year, and in the raceme inflorescence of the male flower, one female flower exists, which produces a large green cylindrical fruits called gourds, with spongy endocarp and about 30 flat and have been used for medicinal purposes and as piscicides [15, 16]

The phytochemistry analysis of the fruit extract showed that it contains 11 major constituents which are; Phenols, Tanins, Alkaloids, Flavonoids, Saponins, Oxalate, Cyanogenic glycoside, Anthraquionols, Steroids, Terpenoids and Phytate with alkaloids and saponins being the dominant components. Piscicidal plants contain different

active ingredients known as alkaloids such as nicotine, pyrethrum, ryania, rotenone, coumerin, resin, akuammine, tannins, saponins and diosgenin [17, 18]. However, these alkaloids are toxic to fish and other aquatic organisms at high concentrations and wear off within a short time [19, 20].

Use of biomarkers such as histological parameters, proximate composition, and haematological parameters are good indicators of fish health. Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as effective and sensitive indicators to check physiological and pathological changes in fishes [21]. The count of red blood cells is relatively a steady indicator and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. In recent years, haematological variables were used more often when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment [21]. As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of fish species following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. [21, 22]. Besides, this type of index reflects certain ecological changes in the environment [23]. Studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the haematological parameters [24]. Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases [25, 26]. Like in warm blooded animals, changes in the blood parameters of fish, which occur because of injuries of the latter organs or tissues, can be used to determine and confirm the dysfunction of the organs or tissues. However in fish, these parameters are more related to the response of the whole organism, that is, to the effect on fish survival, reproduction and growth. It should be noted that although the mechanisms of fish physiology and biochemical reaction to toxicants has not been investigated enough, it is obvious that species differences of these mechanisms exist. Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components [27]. Thus, water quality is one of the major factors, responsible for individual variations in fish haematology.

Audu *et al*. [7] studied the changes in haematological parameters of *Clarias gariepinus* exposed to sublethal concentrations of (0.125, 0.0625, 0.0313, 0.0156 and 0.0080 mg/L) century plant (*Agave americana*) leaf dust for 28 days. From their study, the mean value of packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cells (RBC) were found to significantly ($P < 0.05$) decrease as the concentration of *A. americana* leaf dust increased while that of white blood cells (WBC) showed significant ($P < 0.05$) increase as the concentration of *A. americana* leaf dust increased. In another study on the toxicity of Aqueous Extract of Desert Date (*Balanites aegyptiaca*) on the juveniles of *Clarias gariepinus* by Absalom *et al*., [28], they found out that haematological parameters such as haemoglobin and haematocrit levels increased in the test medium when compared to the control groups and they concluded that the extract of the plant is toxic to *Clarias gariepinus* and therefore the use of the fruit extract could lead to contamination and disruption of the ecological system, thus posing great threat to fish and other non-target organisms in the aquatic ecosystem. On analysis of some haematological parameters of the test organisms, they noticed that; haemoglobin concentrations of treated fishes irrespective of their developmental stages were markedly lower when compared to the control. In the work of Ubaha *et al.* [29] with *Hypoestes forskalei*, the PCV and RBC counts of all treated fishes irrespective of their developmental stage; were lower than the control while the WBC counts recorded in most of the treated adult fishes were lower than those recorded in fingerlings and juvenile fishes treated with corresponding concentration of the plant extract. They concluded that fishes killed by the application of this extract to water body, should not be consumed due to the tendency of the extract to induce long term health hazards on the internal organs of the consumers.

MATERIALS AND METHODS

Collection of Experimental Organisms and Plant

A total number of two hundred (200) fish were used in this experiment. The juveniles of *Clarias gariepinus* with mean weight of 21.72 ± 3.63g and length of 15.12 ± 1.01cm respectively were obtained from a private fish farm at Aluu, Port Harcourt, Nigeria. The juveniles were carefully transported in the evening in a plastic container to

the laboratory of Animal and Environmental Biology, University of Port Harcourt, the fish were acclimatized for 14 days using 100 L circular plastic tanks in the laboratory. During the acclimatization and trial periods, the fish were fed with Coppens feed. Similarly, during this period, the 50% of the water in the acclimatization tanks were siphoned and replaced with fresh water stored in storage tank in the laboratory to remove feed leftovers and faecal deposits

The fruits of *L. Cylindrical* were collected from the Choba Campus of the University. Identification of the plant was done by a technical staff of the Department of Plant Science and Biotechnology. The fruits were homogenized using Binatone electric blender without adding water and sieved with 30μm mesh size. The sub-lethal concentrations of *L. cylindrica* fruit extract was achieved after series of preliminary and acute toxicity tests. O.00 mg/L, 0883 mg/L, 1766 mg/L, 3532 mg/L and 7063 mg/L) were concentrations used for the exposure.

A total of 15 plastic containers were used with 3 replicates per treatment with 10 fish randomly exposed to the different concentrations of *Luffa cylindrica* extract for eight (8) weeks. During this period, freshly prepared test solution was added on a regular basis to maintain the concentration level after the waste was removed.

During the 56-day experimental period, some physico-chemical parameters such as pH, dissolved oxygen (DO), Conductivity, Total dissolved solid and temperature were monitored using standard procedures as described by APHA, [30] on a weekly basis.

Determination of Haematological Parameters.
Two fish were sacrificed every two weeks (14 days) and blood collected using insulin syringe and needle rinsed with EDTA to determine the various haematological parameters [31]. The blood collected was transferred into a 5ml heparimized (EDTA bottle) tube and held in ice pending analysis and the following haematological parameters: Red Blood Cell Counts (RBC), White Blood Cells Count (WBC), Packed Cells Volume (PCV), Mean Corpuscular Volume (MCV), Haemoglobin and Platelet Count were determined using the method described by [31].

Data Analysis
The data from the physico-chemical and haematological parameters were subjected to analysis by calculating means, standard error and Analysis of Variance (ANOVA) at 5% probability level.

Statistically data from the physico-chemical and haematological parameters were subjected to analysis by using one way ANOVA and Duncan's multiple range tests to compare the mean values of the samples and to avoid error inherent in performing multiple *t*-tests. Results were tested for statistically significant differences at 0.05 levels. All statistics were done with the aid of IBM SPSS version 20.0. Results were presented in mean ± standard deviation.

RESULTS
Ecological Parameters
The pH of the test media was slightly acidic which ranged from 6.63±0.38 to 5.95±0.40. This indicate that *L. cylindrica* fruit extracts increased the acidity of the water although the differences were not significant ($p>0.05$). The temperature ranges from 27.07±0.31^{0}C to 27.48±0.62^{0}C and the differences in the temperature was not also statistically significant ($F= 0.32$, $P>0.05$). The Dissolved Oxygen (DO) for the different concentrations during this study showed a decreasing trend; 0.00 mg/L (6.10±0.20 mg/L), 883 mg/L (5.10±0.28 mg/L), 1766 mg/L (4.98±0.28 mg/L), 3532 mg/L (4.38±0.56 mg/L) and 7063 mg/L (4.18±0.50mg/L), and the differences in the DO values were statistically significant ($p <0.05$) but were within WHO/FMEVN standard.

The TDS and Conductivity showed an increasing tendency, the TDS values obtained for the different concentrations of fruit extract of *L. cylindrica* indicated that there was significant differences across the different concentrations ($P<0.05$). The control recorded the least value of 9.97±1.85 mg/L while the 7063 mg/L concentration which was the highest concentration had a value of 34.23±5.53 mg/L. The result for the conductivity showed that the values obtained across the treatments were within WHO/FMEVN standard. The differences in conductivity values were statistically significant ($p < 0.05$).

Red Blood Cell (Erythrocyte) Counts.
The red blood cell counts (RBC) of *Clarias gariepinus* juveniles exposed to sublethal concentration of fruit extract of *Luffa cylindrica* is presented in Fig 1. In the study, it was found that, the mean red blood cell counts of the exposed fish decreased with increased concentration of the extract although the ANOVA revealed between the two highest concentrations and the control that there was no statistical difference ($P < 0.05$).

White Blood Cell (Leucocytes) Counts
The white blood cell counts (WBC) of *C. gariepinus* fingerlings exposed to sublethal concentrations of

fruit extract *L. cylindrica* is also presented in fig 2. The result shows that the white blood cell counts of the exposed fish increased with increased concentration of the extract and this increase was statistically different between the two highest concentrations and the control ($p > 0.05$).

Packed Cells Volume (PCV)

The mean PCV values of *C. gariepinus* juveniles exposed to the different sublethal concentrations of the fruit extract of *L. cylindrica* shows that the PCV values were decreasing with increase in concentration and the differences in mean PCV values obtained across the different concentrations was statistically significant between the control and the three highest concentrations ($P<0.05$) as shown in fig 3.

Mean Corpuscular Volume (MCV)

Figure 4 shows the mean corpuscular volume (MCV) for *C. gariepinus* exposed to all the test concentration of *L. cyndrica* extract. An increase was observed in this parameter with increase in concentration of fruit extract of *L. cylindrica*. The differences in MCV values were not statistically significant ($F = 1.25$, $P > 0.05$).

Haemoglobin (Hb) Concentration

The result for haemoglobin of the test organism is also presented in fig. 5. The result shows that the haemoglobin of the test organisms decreases with increase in concentration, the control having the highest value of haemoglobin, the differences in the haemoglobin values of the test organism exposed to the fruit extract of *L. cylindrica* was statistically significant ($p < 0.05$).

Blood Platelets of Test Organisms

This parameter showed a declining trend with increase in concentration, the control having the highest platelet value of 87.75 ± 6.34 and the highest concentration 7063 mg/L having a value of 58.75 ± 6.29. The differences in the platelet values across the different concentrations were also statistically significant ($P < 0.05$) as shown in fig 6.

DISCUSSION

In the course of this study, some water quality indicators were monitored; the results shown that *L. cylindrica* did not affect the temperature in the different concentrations. Ecological parameters such as temperature, pH, dissolved oxygen, electric conductivity and total dissolved solids are vital indicators of the state of health of aquatic lives [7]. In this study, the observed parameters were noted to be significantly different from the Control except for temperature and pH. The pH of the water samples varied from concentration to concentration and the values obtained for the different treatments were lower than the standard given by WHO [32] for fish survival. WHO/FMEVN (2003) recommended pH range of 6.5 to 8.5 for fresh water fishes, the decline in pH with time may be due to the production of acidic products of metabolism [33] by the plant material in water. Electrical conductivity and TDS also increased across the different treatments, this may be due to the chemical composition of *L. cylindrica* [34]. DO is one of the most important factor for all living organisms especially fish survival [35]. As revealed from this study, DO of the water samples were decreased by increasing concentration of *L. cylindrica.* Prasad *et al*. [36] reported that the reduction in dissolved oxygen content in a bioassay media as toxicant concentration increased may be due to antioxidant property of the toxicant.

The physico-chemical parameters monitored in this study tend to have contributed little or none to the toxicity of *L. cylindrica* fruit extract.

The sub-lethal toxicity test carried out showed that *L. cylindrical* fruit extract caused significant changes in the haematological indices of *C. gariepinus*. The changes in the values of the haematological indices of the test fish is similar to that reported by Ayotunde *et al*. [37], Audu *et al*. [7]. The packed cell volume, haemoglobin and erythrocyte counts are good indicators of oxygen transportation capacity of fish thus making it possible to establish relationship with the oxygen concentration available in the habitat and the health status of the fish [38]. On the other hand, the white blood cells confer protection against infectious agent caused by microbial and chemical factors [39].

The significant reduction in PCV, Hb, RBC, MCV, Platelets could be indication of severe anaemia caused by destruction of erythrocytes [10] or haemo-dilution [40], resulting from impaired osmoregulation across the gill epithelium as there was significant decrease in dissolved oxygen level. PCV is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood. Larson *et al,* [41]; Adamu and Audu, [42] reported significant decrease in PCV and attributed damage and impaired osmoregulation of the gill causing anaemia and haemodilution. Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as good indicator of anaemia [7]. Reduction in Hb content of treated *C. gariepinus* may be an indication of decline in haemoglobin synthesis as well as reduction in oxygen carrying capacity which may perhaps be as a result of interference of the extract with haemoglobin synthesis pathway. The Hb values fall lower than the range reported for catfish [37, 43].

The reduction may be due to increased rate of breakdown of red blood cells and/or reduction in the rate of formation of red blood cells [44] which may probably have been caused by the plant extract. Significant reduction in Hb content and erythrocyte count in the blood of a freshwater fish, *Clarias gariepinus,* on exposure to an aqueous leaves extracts of *Lepidagathis alopecuroides* had been reported [45].

Haemoglobin concentration and packed cell volume values are directly correlated to erythrocytes count which may be due to the synergistic linkage of the blood cells. The increase in the white blood cells may have been induced as protection against disease and improving the health mechanism of the fish in the stressed condition. Gafaar *et al.* [46] reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants. Changes in the values of haematological parameters in these studies were concentration- dependent. This may be due to the level as well as duration of exposure to the toxicant.

Table 1: Physico-Chemical Parameters of sublethal concentrations of *L. cylindrica* fruit extract

Parameters	Control	Treatment1 7063 mg/L	Treatment2 3532 mg/L	Treatment3 1766 mg/L	Treatment4 883mg/L	WHO/FMEVN Standard
pH	6.63±0.38^{a}	6.35±0.21^{ab}	6.08±0.17^{a}	5.98±0.17^{b}	5.95±0.40^{b}	6.5 – 8.5
DO (mg/L)	6.10±0.20^{a}	5.10±0.28^{ab}	4.98±0.28^{b}	4.38±0.56^{b}	4.18±0.50^{b}	> 4.0
Temperature (^{0}C)	27.07±0.31^{a}	27.45±0.78^{a}	27.40±0.56^{a}	27.48±0.62^{a}	27.48±0.61^{a}	20 – 30
TDS (mg/L)	9.97±1.05^{d}	12.45±1.34^{d}	17.55±1.57^{cd}	21.98±1.84^{bc}	34.23±5.53^{a}	250
Conductivity (uS/cm)	22.83±7.52^{c}	21.50±7.78^{c}	35.15±3.71^{b}	33.13±2.89^{b}	48.63±5.16^{a}	100

Note: Values in each row with the same superscript are not significantly different at P >0.05

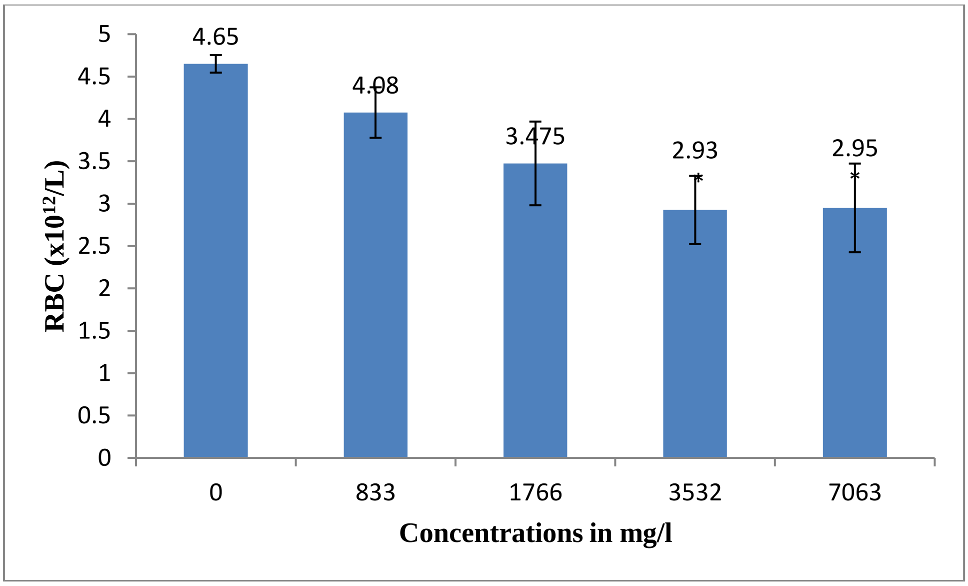

Fig 1: Red Blood Cell level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = p <0.05 compared to control

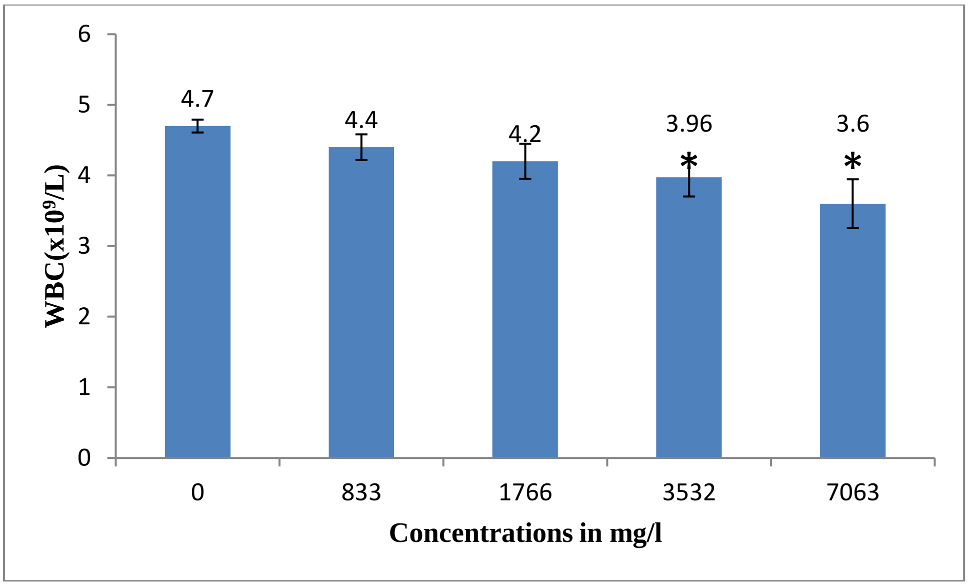

Fig 2: White Blood Cell level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = $p<0.05$ compared to control.

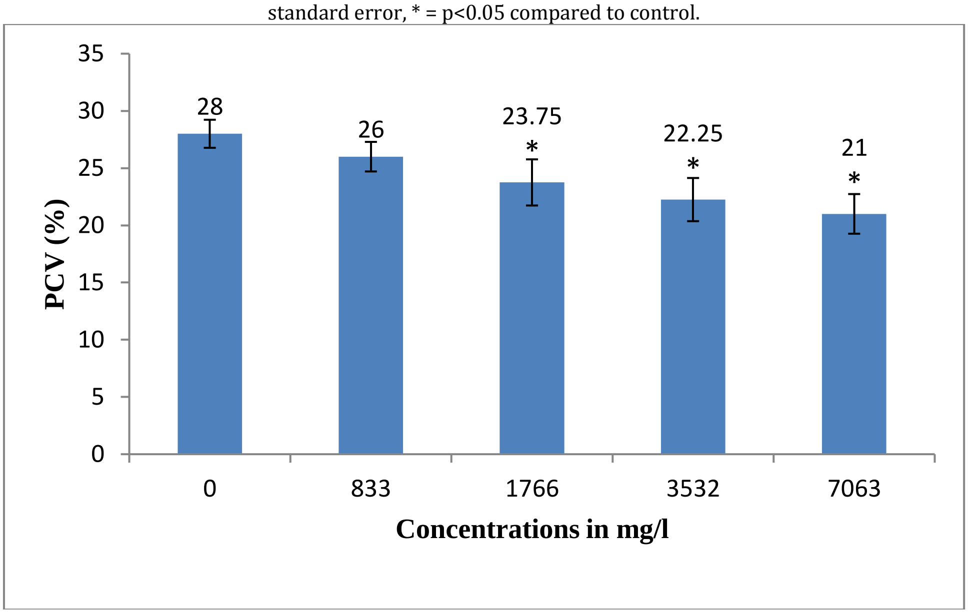

Fig 3: Packed cell volume level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = $p<0.05$ compared to control

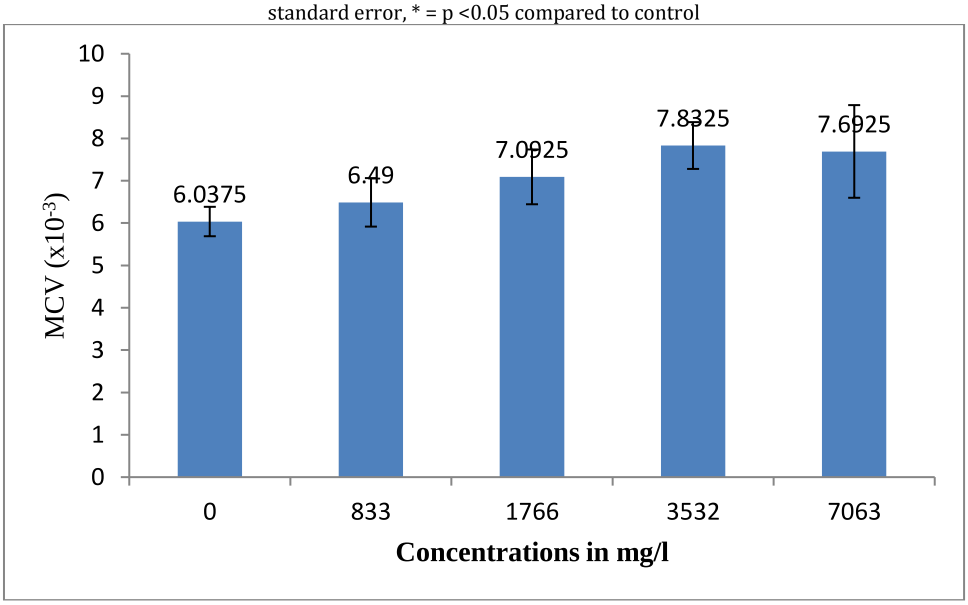

Fig 4: Mean corpuscular volume level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = p<0.05 compared to control

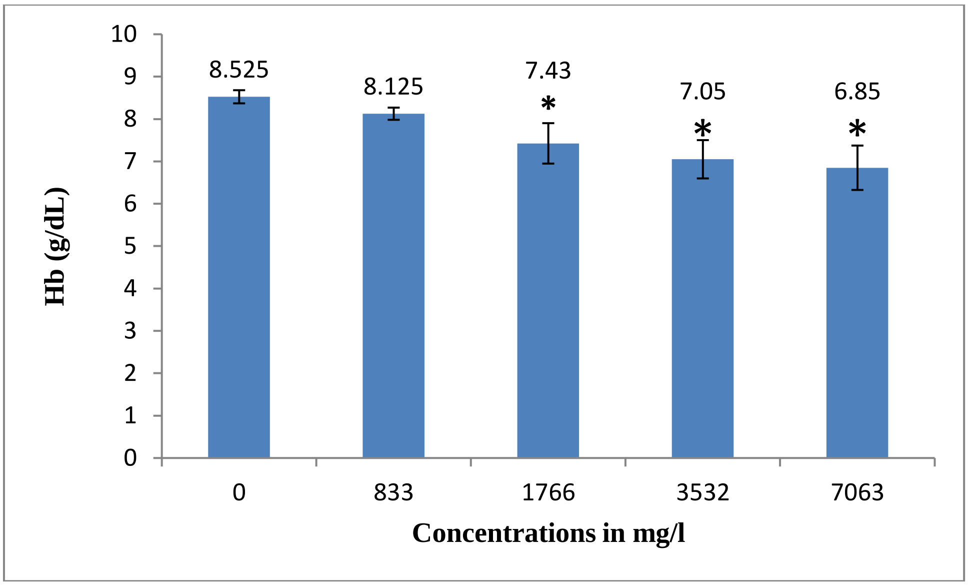

Fig 5: Haemoglobin level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = p <0.05 compared to control

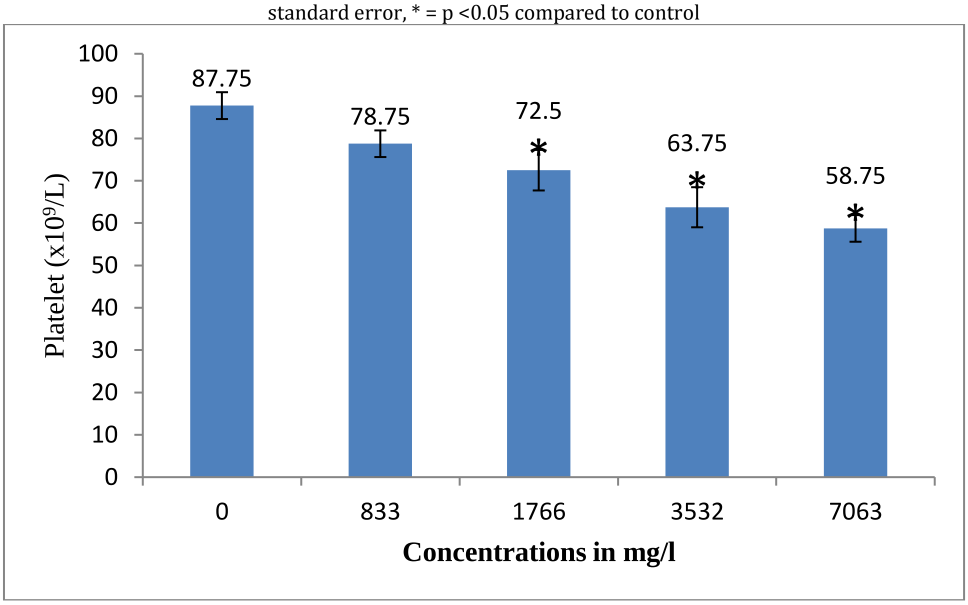

Fig 6: Platelet level of *C. gariepinus* exposed to concentrations of *L. cylindrica* fruit extract for 56 days. Vertical bars represents standard error, * = p <0.05 compared to control

CONCLUSION

The fruit extract of *L. cylindrica* caused negative changes in the haematological parameters of *C. garienpinus* by decreasing the platelet, red blood cell and packed cell volume, MCV while the white blood cell increased in trying to fight the changes in the system.

Water physico-chemical parameters were also affected by the fruit extract and led to stress factors that were reflected in behaviours distress like restlessness, increased respiratory rate, gasping for air and loss of balance of the test fish. Consequently, the continuous use of the fruit extract of *L. cylindrica* could lead to contamination of the aquatic system, thus posing great threat to fish and other non-target organisms. Therefore, monitoring the haematological changes in the African sharp-tooth catfish (*Clarias gariepinus*) will assist in evaluating aquatic pollution, fish health and fish quality in rivers and other water bodies.

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